

APPENDIX 1
PATENTS THAT INTERFERE WITH APPLICATION

A. U.S. Patent No. 4,716,106

The '106 Patent issued on December 29, 1987, to David J. Chiswell for "Detecting Polynucleotide Sequences." It issued from Application No. 706,747, filed July 23, 1985, which claims the benefit of a British application filed March 1, 1984. Amersham International is the assignee named on the face of the '106 Patent. Pergolizzi first informed the Examiner that the '106 Patent interferes with the Pergolizzi Application in Pergolizzi's Amendment of July 25, 1997.

B. U.S. Patent No. 4,882,269

The '269 Patent issued on November 21, 1989, to Robert J. Schneider and Thomas E. Shenk for an "Amplified Hybridization Assay." It issued from Application No. 06/940,712, filed December 11, 1986, which is a continuation-in-part of Application No. 06/808,695, filed December 13, 1985. Princeton University is the assignee named on the face of the '269 Patent. Pergolizzi first informed the Examiner that the '269 Patent interferes with the Pergolizzi Application in Pergolizzi's Amendment of July 25, 1997.

C. U.S. Patent No. 5,424,188

The '188 Patent issued on June 13, 1995, to Robert J. Schneider and Thomas E. Shenk for an "Amplified Hybridization Assay." It issued from Application No. 07/963,923, filed October 20, 1992, which is a continuation of Application No. 07/400,831, filed August 29, 1989, which is a divisional of Application No. 06/940,712 (the '269 Patent), filed December 11, 1986, which is a

continuation-in-part of Application No. 06/808,695, filed December 13, 1985. Princeton University is the assignee named on the face of the '188 Patent. The '188 Patent is terminally disclaimed over the '269 Patent.

D. US Patent No. 5,124,246

The '246 Patent issued on June 23, 1992, to Michael S. Urdea, Brian Warner, and Thomas Horn for "Nucleic Acid Multimers and Amplified Nucleic Acid Hybridization Assays Using Same." The '246 Patent issued from Application No. 340,031, filed April 18, 1989, which is a continuation-in-part of Application No. 252,638, filed September 30, 1988, which is a continuation-in-part of Application No. 185,201, filed April 22, 1988, which is a continuation-in-part of Application No. 109,282, filed October 15, 1987. Chiron Corporation is the assignee named on the face of the '246 Patent. Pergolizzi first apprised the Examiner of the existence of the '246 Patent in Pergolizzi's Amendment of March 5, 1996.

APPENDIX 2

PROPOSED COUNTS

Proposed Count 1

A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of

(a) a labelled polynucleotide secondary probe having a complex single-stranded polynucleotide sequence, and

(b) a polynucleotide primary probe having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of

(i) contacting the sample under hybridisation conditions with the primary probe,

(ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and

(iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.

Proposed Count 2

A method for the detection of a target nucleotide sequence, comprising:

(a) contacting the target nucleotide under conditions that permit hybridization with

(i) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence, and

(ii) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal generating component and a polymer capable of binding to a different portion of the tail of the primary probe; and

(b) detecting the amplified signal generated by a reaction product formed in step (a), in which the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and a plurality of secondary probes are bound to different portions of the primary probe tail.

Proposed Count 3

A hybridization assay kit for the detection of a target nucleotide sequence, comprising:

(a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and

(b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a non-radioactive signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.

Proposed Count 4

A nucleic acid hybridization assay wherein:

I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:

(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and

(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;

II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;

III. unbound multimer is removed;

IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;

V. unbound labeled oligonucleotide is removed; and

VI. the presence of label bound to the multimer is detected.

APPENDIX 3

COMPARISON OF COUNTS WITH REPRESENTATIVE PATENT CLAIMS

<u>Count 3 (Claim 25 of US4882269)</u>	<u>Claim 49 of US4882269</u>
<p>A hybridization assay kit for the detection of a target nucleotide sequence, comprising:</p> <p>(a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and</p> <p>(b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a non-radioactive signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.</p>	<p>A hybridization assay kit for the detection of a target nucleotide sequence, comprising:</p> <p>(a) a primary probe cassette which comprises a cloning vector having</p> <p>(i) a multiple cloning site into which a target nucleotide sequence can be inserted and cloned and</p> <p>(ii) nucleotide sequences which are capable of hybridizing to their complements which comprise a plurality of secondary probes; and</p> <p>(b) the plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal-generating component and a nucleotide sequence capable of hybridizing to a different portion of the portion of the primary probe described in (a)(ii), which provides for the generation of an amplified signal when the plurality of secondary probes are hybridized to different portions of the portion of the primary probe described in (a)(ii).</p>
<u>Count 3 (Claim 25 of US4882269)</u>	<u>Claim 1 of US5424188</u>
<p>A hybridization assay kit for the detection of a target nucleotide sequence, comprising:</p> <p>(a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and</p> <p>(b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a non-radioactive signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the</p>	<p>A hybridization assay kit for the detection of a target nucleotide sequence in a sample which target is hybridized to a primary probe, which primary probe has</p> <p>(1) a polynucleotide sequence complementary to the target nucleotide sequence and</p> <p>(2) a polymeric tail with a plurality of binding sites, each site incapable of binding to the target sequence and capable of binding a member of a family of secondary probes, which kit comprises:</p> <p>a plurality of secondary probes comprising a family of signal-generating probes, each member of the family having at least (1) a signal-generating</p>

<p>plurality of secondary probes are bound to different portions of the primary probe tail.</p>	<p>component and (2) a polymer capable of binding to a distinct binding site of the tail of the primary probe which site is not bound by other members of the family; which kit provides for the generation of an amplified signal when the plurality of secondary probes are bound to distinct binding sites of the tail of the primary probe</p>
<p><u>Count 4 (Claim 53 of US5124246)</u></p>	<p><u>Claim 39 of US5124246</u></p>
<p>A nucleic acid hybridization assay wherein:</p> <p>I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:</p> <p>(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and</p> <p>(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;</p> <p>II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;</p> <p>III. unbound multimer is removed;</p> <p>IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;</p> <p>V. unbound labeled oligonucleotide is removed; and</p> <p>VI. the presence of label bound to the multimer is detected.</p>	<p>A synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:</p> <p>(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and</p> <p>(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds.</p>

APPENDIX 4

COMPARISON OF COUNTS WITH APPLICATION CLAIMS

<u>Count 1 (Claim 1 of the US4716106)</u>	<u>Claims 443 and 532-35 of Pergolizzi Application</u>
<p>A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of</p> <p>(a) a labelled polynucleotide secondary probe having a complex single-stranded polynucleotide sequence, and</p> <p>(b) a polynucleotide primary probe having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of</p> <p>(i) contacting the sample under hybridisation conditions with the primary probe,</p> <p>(ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and</p> <p>(iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.</p>	<p>443. A process for detecting an analyte having one or more molecularly recognizable portions thereon, comprising:</p> <p>providing a composition of matter comprising:</p> <p>a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and</p> <p>a second part which comprises one or more signalling entities substantially incapable of binding to or hybridizing with the molecularly recognizable portion on said analyte, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable of providing a detectable signal;</p> <p>forming a complex comprising said composition and said analyte; and</p> <p>detecting said analyte by a signal provided by said signal generating portion or portions present in said complex.</p> <p>532. The process of claim 443, wherein said analyte is a DNA sequence, said bridging entity is a single-stranded DNA sequence, and said signalling entities are single-stranded DNA sequences.</p> <p>533. The process of claim 532, wherein said bridging entity is derived from a filamentous phage.</p> <p>534. The process of claim 533, wherein said signalling entities are derived from filamentous phages.</p> <p>535. The process of claim 534, wherein said bridging entity codes for a gene product or</p>

	<p>fragment thereof, and said forming step comprises either (i) contacting said analyte with said bridging entity to form a first complex and thereafter contacting said first complex with said signalling entities to form said detectable complex or (ii) contacting said bridging entity with said signalling entities to form a first complex and thereafter contacting said first complex with said analyte to form said detectable complex.</p>
<u>Count 2 (Claim 1 of the US4882269)</u>	<u>Claims 443 and 536-37 of Pergolizzi</u>
<p>A method for the detection of a target nucleotide sequence, comprising:</p> <p>(a) contacting the target nucleotide under conditions that permit hybridization with</p> <p>(i) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence, and</p> <p>(ii) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal generating component and a polymer capable of binding to a different portion of the tail of the primary probe; and</p> <p>(b) detecting the amplified signal generated by a reaction product formed in step (a), in which the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and a plurality of secondary probes are bound to different portions of the primary probe tail.</p>	<p><u>Application</u></p> <p>443. A process for detecting an analyte having one or more molecularly recognizable portions thereon, comprising:</p> <p>providing a composition of matter comprising:</p> <p>a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and</p> <p>a second part which comprises one or more signalling entities substantially incapable of binding to or hybridizing with the molecularly recognizable portion on said analyte, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable of providing a detectable signal;</p> <p>forming a complex comprising said composition and said analyte; and</p> <p>detecting said analyte by a signal provided by said signal generating portion or portions present in said complex.</p> <p>536. The process of claim 443, wherein said analyte is a polynucleotide, said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and said one</p>

	<p>or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte.</p> <p>537. The process of claim 536, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.</p>
<u>Count 3 (Claim 25 of the US4882269)</u>	<u>Claims 411, 538-39, 546 and 547 of Pergolizzi Application</u>
<p>A hybridization assay kit for the detection of a target nucleotide sequence, comprising:</p> <p>(a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and</p> <p>(b) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a non-radioactive signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.</p>	<p>411. A kit for the detection in a sample of an analyte having one or more molecularly recognizable portions thereon, comprising as components thereof:</p> <p>(i) a container carrying a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with said molecularly recognizable portion on said analyte, and a second portion comprising one, or more nucleic acid sequences or segments; and</p> <p>(ii) a container carrying more than one non-radioactive signalling entity, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity second portion nucleic acid sequence or segment, and one or more signal generating portions, each such portion being capable of providing a detectable signal.</p> <p>538. The kit of claim 411, wherein said analyte is a polynucleotide, said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte.</p> <p>539. The kit of claim 538, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.</p> <p>546. A kit for the detection in a sample of an analyte having one or more molecularly recognizable portions thereon, comprising as components thereof:</p>

	<p>(i) a container carrying a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with said molecularly recognizable portion on said analyte, and a second portion comprising one, or more nucleic acid sequences or segments; and</p> <p>(ii) a container carrying more than one signalling entity, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity second portion nucleic acid sequence or segment, and one or more signal generating portions, each such portion being capable of providing a detectable signal, and</p> <p>wherein said analyte is a polynucleotide, said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte.</p> <p>547. The kit of claim 546, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.</p>
<p><u>Count 4 (Claim 53 of the US5124246)</u></p>	<p><u>Claims 443 and 540-41 of Pergolizzi</u> <u>Application</u></p>
<p>A nucleic acid hybridization assay wherein:</p> <p>I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:</p> <p>(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and</p> <p>(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled</p>	<p>443. A process for detecting an analyte having one or more molecularly recognizable portions thereon, comprising:</p> <p>providing a composition of matter comprising:</p> <p>a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and</p> <p>a second part which comprises one or more signalling entities substantially incapable of binding to or hybridizing with the molecularly recognizable portion on said</p>

<p>oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;</p> <p>II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;</p> <p>III. unbound multimer is removed;</p> <p>IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;</p> <p>V. unbound labeled oligonucleotide is removed; and</p> <p>VI. the presence of label bound to the multimer is detected.</p>	<p>analyte, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable of providing a detectable signal; forming a complex comprising said composition and said analyte; and detecting said analyte by a signal provided by said signal generating portion or portions present in said complex.</p> <p>540. The process of claim 443, wherein said analyte is a single-stranded DNA sequence fixed to a solid support, said bridging entity comprises non-naturally occurring or artificially modified DNA, said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, said bridging entity first portion is covalently bound to said bridging entity second portion, and said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.</p> <p>541. The process of claim 540, wherein said signalling entities are single-stranded oligo- or polynucleotide sequences, said bridging entity first portion is capable of encoding a gene product or fragment thereof, and the process further comprises one or more washing steps prior to detection.</p>
<p><u>Count 4 (Claim 53 of the US5124246)</u></p>	<p><u>Claims 283 and 542-43 of Pergolizzi Application</u></p>
<p>A nucleic acid hybridization assay wherein:</p> <p>I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:</p> <p>(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and</p> <p>(b) a multiplicity of second single-stranded oligonucleotide units all of which</p>	<p>283. A composition of matter comprising:</p> <p>a first part which comprises a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with a molecularly recognizable portion on an analyte, and a second portion comprising one or more nucleic acid sequences or segments; and</p> <p>a second part which comprises one or more non-radioactive signalling entities substantially incapable of binding to or hybridizing with the molecularly recognizable portion on said analyte, each such entity</p>

<p>are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;</p> <p>II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;</p> <p>III. unbound multimer is removed;</p> <p>IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;</p> <p>V. unbound labeled oligonucleotide is removed; and</p> <p>VI. the presence of label bound to the multimer is detected.</p>	<p>comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity nucleic acid second portion, and one or more signal generating portions capable of providing a detectable signal.</p> <p>542. The composition of claim 283, wherein said analyte is a single-stranded DNA sequence fixed to a solid support, said bridging entity comprises non-naturally occurring or artificially modified DNA, said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, said bridging entity first portion is covalently bound to said bridging entity second portion, and said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.</p> <p>543. The composition of claim 542, wherein said signalling entities are single-stranded oligo- or polynucleotide sequences and said bridging entity first portion is capable of encoding a gene product or fragment thereof.</p>
<p><u>Count 4 (Claim 53 of the US5124246)</u></p>	<p><u>Claims 411 and 544-45 of Pergolizzi Application</u></p>
<p>A nucleic acid hybridization assay wherein:</p> <p>I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:</p> <p>(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and</p> <p>(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent</p>	<p>411. A kit for the detection in a sample of an analyte having one or more molecularly recognizable portions thereon, comprising as components thereof:</p> <p>(i) a container carrying a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with said molecularly recognizable portion on said analyte, and a second portion comprising one, or more nucleic acid sequences or segments; and</p> <p>(ii) a container carrying more than one non-radioactive signalling entity, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity second portion nucleic acid sequences or segments, and one or more signal generating portions, each such portion being capable of providing a detectable signal.</p> <p>544. The kit of claim 411, wherein said</p>

bonds;

II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;

III. unbound multimer is removed;

IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;

V. unbound labeled oligonucleotide is removed; and

VI. the presence of label bound to the multimer is detected.

analyte is a single-stranded DNA sequence fixed to a solid support, said bridging entity comprises non-naturally occurring or artificially modified DNA, said bridging entity first portion comprises a linear single-stranded polynucleotide sequence, said bridging entity first portion is covalently bound to said bridging entity second portion, and said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.

545. The kit of claim 544, wherein said signalling entities are single-stranded oligo- or polynucleotide sequences and said bridging entity first portion is capable of encoding a gene product or fragment thereof.

APPENDIX 5
SUPPORT FOR APPLICATION CLAIMS 532-47

<u>Pergolizzi Process Claims 532-35</u>	<u>Support in Pergolizzi Application</u>
<p>532. The process of claim 443, wherein *said analyte is a DNA sequence,</p> <p>**said bridging entity is a single-stranded DNA sequence, and</p> <p>***said signalling entities are single-stranded DNA sequences.</p>	<p>*Original Claims 4, 7, 69, 102 and 147 (as filed May 5, 1983). Pergolizzi Specification p.10, 2nd para.; p.11, 2nd para.; p.11, 2nd para.; p.12, 2nd para.; p.13, 4th para.; p.27, 3rd para.; p.29, 2nd para.; p.30, 1st and 2nd paras.; Fig.2.</p> <p>**Original Claims 7, 20, 29, 30, 69, 125 and 147. Pergolizzi Specification p.10, 2nd para.; p.17, 2nd para.; pp.57-58; Fig.2.</p> <p>***Original Claims 43 and 134. Pergolizzi Specification pp.57-58; Fig.2.</p>
<p>533. The process of claim 532, wherein said bridging entity is derived from a filamentous phage.</p>	<p>Original Claims 31, 32, 70, 111, 126-27 and 148. Pergolizzi Specification p.16, 2nd para; pp.57-61.</p>
<p>534. The process of claim 533, wherein said signalling entities are derived from filamentous phages.</p>	<p>Original Claims 39, 44, 45, 67, 71, 135-36 and 149. Pergolizzi Specification p.16, 2nd para; pp.57-61.</p>
<p>535. The process of claim 534, wherein *said bridging entity codes for a gene product or fragment thereof, and</p> <p>**said forming step comprises either (i) contacting said analyte with said bridging entity to form a first complex and thereafter contacting said first complex with said signalling entities to form said detectable complex or (ii) contacting said bridging entity with said signalling entities to form a first complex and thereafter contacting said first complex with said analyte to form said detectable complex.</p>	<p>*Original Claims 16 and 111. Pergolizzi Specification p.14, 3rd para.; p.17, 2nd para.; p.30, 2nd and 3rd paras.</p> <p>**Pergolizzi Specification p.28, 2nd para.; p.31 4th para. to p.34, 1st para.; Figs.1-2.</p>
<u>Pergolizzi Process Claims 536-37</u>	<u>Support in Pergolizzi Application</u>
<p>536. The process of claim 443, wherein *said analyte is a polynucleotide,</p>	<p>*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.10, 2nd para.; p.11, 2nd para.; p.11, 2nd para.; p.12, 2nd para.; p.13, 4th</p>

<p>**said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and</p> <p>***and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte.</p>	<p>para.; p.27, 3rd para.; p.29, 2nd para.; p.30, 1st and 2nd paras.; Fig.2.</p> <p>**Original Claims 18 and 113. Pergolizzi Specification p.10, 2nd para.; p.15, 1st para.; p.17, 2nd para.; p.27, 1st para.; Fig.2.</p> <p>***Pergolizzi Specification p.14, 2nd para; Fig.2.</p>
<p>537. The process of claim 536, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.</p>	<p>Pergolizzi Specification p.4, 2nd para; p.6, 3rd para.; p.16, 1st para.; p.22, 1st para.</p>
<p><u>Pergolizzi Kit Claims 538-39</u></p>	<p><u>Support in Pergolizzi Application</u></p>
<p>538. The kit of claim 411, wherein</p> <p>*said analyte is a polynucleotide,</p> <p>**said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and</p> <p>***and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte.</p>	<p>*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.10, 2nd para.; p.11, 2nd para.; p.11, 2nd para.; p.12, 2nd para.; p.13, 4th para.; p.27, 3rd para.; p.29, 2nd para.; p.30, 1st and 2nd paras.; Fig.2.</p> <p>**Original Claims 18 and 113. Pergolizzi Specification p.10, 2nd para.; p.15, 1st para.; p.17, 2nd para.; p.27, 1st para.; Fig.2.</p> <p>***Pergolizzi Specification p.14, 2nd para; Fig.2.</p>
<p>539. The kit of claim 538, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.</p>	<p>Pergolizzi Specification p.4, 2nd para; p.6, 3rd para.; p.16, 1st para.; p.22, 1st para.; p.22, 1st para.</p>
<p><u>Pergolizzi Method Claims 540-41</u></p>	<p><u>Support in Pergolizzi Application</u></p>
<p>540. The process of claim 443, wherein</p> <p>*said analyte is a single-stranded DNA sequence fixed to a solid support,</p> <p>**said bridging entity comprises non-naturally occurring or artificially modified DNA,</p> <p>***said bridging entity first portion comprises a linear single-stranded polynucleotide sequence,</p> <p>****said bridging entity first portion is</p>	<p>*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.11, 2nd para; p.12, 2nd para.; p.28, 1st para.; p.31, 4th para.</p> <p>**Pergolizzi Specification p.15, 1st and 2nd paras.; p.25, 3rd and 4th paras.; p.27, 1st para.</p> <p>***Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.17, 2nd para..</p> <p>****Original Claims 20 and 115. Pergolizzi Specification p.13, 3rd para; p.15, 2nd para.; p.23,</p>

<p>covalently bound to said bridging entity second portion, and</p> <p>*****said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.</p>	<p>1st para.; p.25, 3rd para.; Fig.2.</p> <p>*****Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.16, 1st para.; p.17, 2nd para.; p.22, 1st para.</p>
<p>541. The process of claim 540, wherein</p> <p>*said signalling entities are single-stranded oligo- or polynucleotide sequences,</p> <p>**said bridging entity first portion is capable of encoding a gene product or fragment thereof, and</p> <p>***the process further comprises one or more washing steps prior to detection.</p>	<p>*Original Claims 43 and 134. Pergolizzi Specification pp.57-58.</p> <p>**Original Claims 16 and 111. Pergolizzi Specification p.14, 3rd para.</p> <p>***Pergolizzi Specification p.18, 1st para.; p.28, 2nd para.</p>
<p><u>Pergolizzi Composition Claims 542-43</u></p>	<p><u>Support in Pergolizzi Application</u></p>
<p>542. The composition of claim 283, wherein</p> <p>*said analyte is a single-stranded DNA sequence fixed to a solid support,</p> <p>**said bridging entity comprises non-naturally occurring or artificially modified DNA,</p> <p>***said bridging entity first portion comprises a linear single-stranded polynucleotide sequence,</p> <p>****said bridging entity first portion is covalently bound to said bridging entity second portion, and</p> <p>*****said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.</p>	<p>*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.11, 2nd para.; p.12, 2nd para.; p.28, 1st para.; p.31, 4th para.</p> <p>**Pergolizzi Specification p.15, 1st and 2nd paras.; p.25, 3rd and 4th paras.; p.27, 1st para.</p> <p>***Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.17, 2nd para..</p> <p>****Original Claims 20 and 115. Pergolizzi Specification p.13, 3rd para.; p.15, 2nd para.; p.23, 1st para.; p.25, 3rd para.; Fig.2.</p> <p>*****Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.16, 1st para.; p.17, 2nd para.; p.22, 1st para.</p>
<p>543. The composition of claim 542, wherein</p> <p>*said signalling entities are single-stranded oligo- or polynucleotide sequences and</p>	<p>*Original Claims 43 and 134. Pergolizzi Specification pp.57-58.</p> <p>**Original Claims 16 and 111. Pergolizzi</p>

<p>**said bridging entity first portion is capable of encoding a gene product or fragment thereof.</p>	<p>Specification p.14, 3rd para.</p>
<p><u>Pergolizzi Kit Claims 544-45</u></p>	<p><u>Support in Pergolizzi Application</u></p>
<p>544. The kit of claim 411, wherein *said analyte is a single-stranded DNA sequence fixed to a solid support,</p> <p>**said bridging entity comprises non-naturally occurring or artificially modified DNA,</p> <p>***said bridging entity first portion comprises a linear single-stranded polynucleotide sequence,</p> <p>****said bridging entity first portion is covalently bound to said bridging entity second portion, and</p> <p>*****said bridging entity second portion is single-stranded and linear and comprises more than one of said nucleic acid sequences or segments.</p>	<p>*Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.11, 2nd para.; p.12, 2nd para.; p.28, 1st para.; p.31, 4th para.</p> <p>**Pergolizzi Specification p.15, 1st and 2nd paras.; p.25, 3rd and 4th paras.; p.27, 1st para.</p> <p>***Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.17, 2nd para..</p> <p>****Original Claims 20 and 115. Pergolizzi Specification p.13, 3rd para.; p.15, 2nd para.; p.23, 1st para.; p.25, 3rd para.; Fig.2.</p> <p>*****Pergolizzi Specification p.10, 2nd para.; p.15, 2nd para.; p.16, 1st para.; p.17, 2nd para.; p.22, 1st para.</p>
<p>545. The kit of claim 544, wherein *said signalling entities are single-stranded oligo- or polynucleotide sequences and</p> <p>**said bridging entity first portion is capable of encoding a gene product or fragment thereof.</p>	<p>*Original Claims 43 and 134. Pergolizzi Specification pp.57-58.</p> <p>**Original Claims 16 and 111. Pergolizzi Specification p.14, 3rd para.</p>
<p><u>Pergolizzi Kit Claims 546 and 547</u></p>	<p><u>Support in Pergolizzi Application</u></p>
<p>546. *A kit for the detection in a sample of an analyte having one or more molecularly recognizable portions thereon, comprising as components thereof:</p> <p>(i) a container carrying a molecular bridging entity comprising a first portion capable of recognizing and binding to or hybridizing with said molecularly recognizable portion on said analyte, and a second portion comprising one, or more nucleic acid sequences or segments; and</p>	<p>*Original Claims 100, 102 and 147. Pergolizzi Specification p.8, last full para.; p.32, 2nd para. through p.33.</p> <p>**Original Claims 4, 7, 69, 102 and 147. Pergolizzi Specification p.10, 2nd para.; p.11, 2nd para.; p.11, 2nd para.; p.12, 2nd para.; p.13, 4th para.; p.27, 3rd para.; p.29, 2nd para.; p.30, 1st and 2nd paras.; Fig.2.</p> <p>***Original Claims 18 and 113. Pergolizzi</p>

<p>(ii) a container carrying more than one signalling entity, each such entity comprising a nucleic acid portion capable of binding to or hybridizing with said bridging entity second portion nucleic acid sequence or segment, and one or more signal generating portions, each such portion being capable of providing a detectable signal,</p> <p>**wherein said analyte is a polynucleotide,</p> <p>***said one or more nucleic acid sequences or segments of said second portion are repeating low-complexity nucleic acid sequences or segments, and</p> <p>****and said one or more nucleic acid sequences or segments of said second portion are incapable of hybridizing to the analyte.</p>	<p>Specification p.10, 2nd para.; p.15, 1st para.; p.17, 2nd para.; p.27, 1st para.; Fig.2.</p> <p>***Pergolizzi Specification p.14, 2nd para; Fig.2.</p>
<p>547. The kit of claim 546, wherein said signal is amplified because the ratio of the signalling entities to the bridging entity exceeds 5.</p>	<p>Pergolizzi Specification p.4, 2nd para; p.6, 3rd para.; p.16, 1st para.; p.22, 1st para.; p.22, 1st para.</p>

APPENDIX 6
APPLICATION CLAIMS PENDING BEFORE
DECEMBER 29, 1988, NOVEMBER 21, 1990, OR JUNE 23, 1993

<u>Claim 1 of US4716106 (Count 1)</u>	<u>Claims 1 and 69-71 of Original Pergolizzi Application</u>
<p>A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of</p> <p>(a) a labelled polynucleotide secondary probe having a complex single-stranded polynucleotide sequence, and</p> <p>(b) a polynucleotide primary probe having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of</p> <p>(i) contacting the sample under hybridisation conditions with the primary probe,</p> <p>(ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and</p> <p>(iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.</p>	<p>1. A method of detecting in a sample an analyte (A) having a molecularly recognizable portion thereon, which comprises:</p> <p>providing a molecular bridging entity (B) having thereon:</p> <p>(i) a portion capable of recognizing said molecularly recognizable portion on said analyte; and</p> <p>(ii) a portion comprising a polynucleotide sequence;</p> <p>and</p> <p>(C) a signalling entity having thereon:</p> <p>(i) a polynucleotide portion capable of annealing to said polynucleotide portion of said bridging entity, thereby to form a stable polynucleotide hybrid, and</p> <p>(ii) a signal generating portion;</p> <p>forming a complex comprising:</p> <p>(1) said analyte (A) complexed through said molecularly recognizable portion to</p> <p>(2) said recognizing portion of said entity (B); said entity (B) being complexed through said polynucleotide portion thereon to</p> <p>(3) said polynucleotide portion of said signalling entity (C);</p> <p>and</p> <p>detecting a signal by means of said signal generating portion present in said complex.</p> <p>69. The method of Claim 1 wherein said recognizable portion on said analyte is a polynucleotide sequence, said recognizing portion on said bridging entity is a polynucleotide sequence capable of stably annealing thereto, said bridging entity is a single-stranded DNA polymer, and said step of detection by means of said signal generating</p>

	<p>portion on said signalling entity is based on non-radioactive detection.</p> <p>70. The method of Claim 69 wherein said bridging entity is derived from a filamentous phage.</p> <p>71. The method of Claim 69 wherein said signalling entity is derived from a filamentous phage.</p>
<u>Claim 1 of US4716106 (Count 1)</u>	<u>Claims 154-56, 159, 161, 165 and 168 of Pergolizzi's Amendment of June 3, 1985</u>
<p>A method of detecting a specific target polynucleotide sequence in a sample, comprising the use of</p> <p>(a) a labelled polynucleotide secondary probe having a complex single-stranded polynucleotide sequence, and</p> <p>(b) a polynucleotide primary probe having a single-stranded sequence complementary to the target sequence and a complex single-stranded sequence complementary to the complex sequence of the secondary probe, which method comprises the steps of</p> <p>(i) contacting the sample under hybridisation conditions with the primary probe,</p> <p>(ii) before, during or after said contact hybridising the labelled secondary probe to the primary probe, and</p> <p>(iii) observing the presence or absence of the label in association with the sample as indicating the presence or absence of the target sequence.</p>	<p>154. A method of detecting in a sample an analyte having a molecularly recognizable portion thereon, comprising:</p> <p>forming a detectable complex comprising</p> <p>(a) said analyte,</p> <p>(b) a non-naturally-occurring molecular bridging entity comprising a portion capable of recognizing and complexing to said molecularly recognizable analyte portion, and a portion comprising a polynucleotide sequence, and</p> <p>(c) a signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity polynucleotide portion and a signal generating portion; and</p> <p>detecting said analyte by a signal provided by said signal generating portion present in said detectable complex.</p> <p>155. The method according to claim 154, characterized in that said forming step comprises contacting said analyte with said bridging entity to form a first complex and contacting said first complex with said signalling entity to form said detectable complex.</p> <p>156. The method according to claim 154, characterized in that said forming step comprises contacting said bridging entity with said signalling entity to form a first complex and contacting said first complex with said analyte to form said detectable complex.</p> <p>159. The method according to claim 158, characterized in that said molecularly recognizable portion on said analyte is selected from the group consisting of an RNA or DNA</p>

	<p>oligo- or polynucleotide sequence, and a protein.</p> <p>161. The method according to claim 154, characterized in that said bridging entity recognizing portion is an RNA or DNA oligo- or polynucleotide sequence.</p> <p>165. The method according to claim 154, characterized in that said bridging entity is selected from the group consisting of a single-stranded, double stranded or partially double-stranded circular DNA polymer, a circular DNA polymer derived from a filamentous phage, an M13 phage or a variant thereof.</p> <p>168. The method according to claim 154, characterized in that said signalling entity is selected from the group consisting of a single stranded, double stranded, or partially double-stranded polynucleotide polymer, a naturally occurring modified DNA, a polynucleotide polymer derived from a T (even) phage, a modified DNA carrying a cloned insert, a polymer derived from a filamentous phage, M13 phage or a variant thereof, and a polymer derived from a circular DNA molecule covalently attached to a non radiolabelled signal generating portion.</p>
<p><u>Claim 1 of US4882269 (Count 2)</u></p>	<p><u>Claims 154 and 161-65 of Pergolizzi's Amendment of June 3, 1985</u></p>
<p>A method for the detection of a target nucleotide sequence, comprising:</p> <p>(a) contacting the target nucleotide under conditions that permit hybridization with</p> <p>(i) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence, and</p> <p>(ii) a plurality of secondary probes comprising a family of signal-generating probes each member of which comprises a signal generating component and a polymer capable of binding to a different portion of the tail of the primary probe; and</p> <p>(b) detecting the amplified signal</p>	<p>154. A method of detecting in a sample an analyte having a molecularly recognizable portion thereon, comprising:</p> <p>forming a detectable complex comprising</p> <p>(a) said analyte,</p> <p>(b) a non-naturally-occurring molecular bridging entity comprising a portion capable of recognizing and complexing to said molecularly recognizable analyte portion, and a portion comprising a polynucleotide sequence, and</p> <p>(c) a signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity polynucleotide portion and a signal generating portion; and</p> <p>detecting said analyte by a signal provided by said signal generating portion</p>

<p>generated by a reaction product formed in step (a), in which the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and a plurality of secondary probes are bound to different portions of the primary probe tail.</p>	<p>present in said detectable complex.</p> <p>159. The method according to claim 158, characterized in that said molecularly recognizable portion on said analyte is selected from the group consisting of an RNA or DNA oligo- or polynucleotide sequence, and a protein.</p> <p>161. The method according to claim 154, characterized in that said bridging entity recognizing portion is an RNA or DNA oligo- or polynucleotide sequence.</p> <p>162. The method according to claim 154, characterized in that said bridging entity polynucleotide portion is a polynucleotide sequence of low complexity.</p> <p>163. The method according to claim 162, characterized in that said bridging entity polynucleotide sequence is selected from the group consisting of a polydeoxy G, polydeoxy C, polydeoxy T or polydeoxy A.</p> <p>164. The method according to claim 161, characterized in that said bridging entity polynucleotide portion and said bridging entity recognizing portion are not capable of hybridizing to identical oligo- and polynucleotide sequences.</p> <p>165. The method according to claim 154, characterized in that said bridging entity is selected from the group consisting of a single-stranded, double stranded or partially double-stranded circular DNA polymer, a circular DNA polymer derived from a filamentous phage, an M13 phage or a variant thereof.</p>
<p><u>Claim 25 of US4882269 (Count 3)</u></p>	<p><u>Claims 100, 102, 137, 138 and 147-48 of Original Pergolizzi Application</u></p>
<p>A hybridization assay kit for the detection of a target nucleotide sequence, comprising:</p> <p>(a) a primary probe which comprises a polynucleotide sequence that is complementary to the target nucleotide sequence and a polymeric tail that has binding sites that are incapable of binding to the target sequence; and</p> <p>(b) a plurality of secondary probes comprising a family of signal-generating probes</p>	<p>100. A kit useful for the detection of an analyte (A) having a molecularly recognizable portion thereon, comprising;</p> <p>I) a carrier being compartmentalized to receive in close confinement therein one or more container means;</p> <p>II) a first container means containing a molecular bridging entity (B) having thereon:</p> <p>(i) a portion capable of recognizing said molecularly recognizable</p>

<p>each member of which comprises a signal-generating component and a polymer capable of binding to a different portion of the tail of the primary probe, which provides for the generation of an amplified signal when the polynucleotide sequence of the primary probe is hybridized to the target nucleotide and the plurality of secondary probes are bound to different portions of the primary probe tail.</p>	<p>portion on said analyte (A); and (ii) a portion comprising a polynucleotide sequence; and (III) a second container means containing a signalling entity (C) having thereon: (i) a polynucleotide portion capable of annealing to said polynucleotide portion of said bridging entity (B) thereby by form a stable polynucleotide hybrid; and (ii) a signal generating portion.</p> <p>102. The kit of Claim 100 wherein said recognizing portion on said bridging entity comprises a polynucleotide sequence.</p> <p>137. The kit of Claim 100 wherein said signal generating portion on said signalling entity is radiolabeled.</p> <p>138. The kit of Claim 100 wherein said signal generating portion on said signalling entity is not radiolabeled.</p> <p>147. The kit of Claim 100 wherein said recognizable portion on said analyte is a polynucleotide sequence, said recognizing portion on said bridging entity is a polynucleotide sequence capable of stably annealing thereto, said bridging entity is a single-stranded DNA polymer, and said signal generating portion on said signalling entity is based on non-radioactive detection.</p> <p>148. The kit of Claim 147 wherein said bridging entity is derived from a filamentous phage.</p>
<p><u>Claim 53 of US5124246 (Count 4)</u></p>	<p><u>Claims 1, 4, 7, 16, 20 and 69 of Original Pergolizzi Application</u></p>
<p>A nucleic acid hybridization assay wherein: I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of: (a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded</p>	<p>1. A method of detecting in a sample an analyte (A) having a molecularly recognizable portion thereon, which comprises: providing a molecular bridging entity (B) having thereon: (i) a portion capable of recognizing said molecularly recognizable portion on said analyte; and (ii) a portion comprising a polynucleotide sequence; and</p>

<p>nucleic acid sequence of interest; and (b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;</p> <p>II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;</p> <p>III. unbound multimer is removed;</p> <p>IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;</p> <p>V. unbound labeled oligonucleotide is removed; and</p> <p>VI. the presence of label bound to the multimer is detected.</p>	<p>(C) a signalling entity having thereon: (i) a polynucleotide portion capable of annealing to said polynucleotide portion of said bridging entity, thereby to form a stable polynucleotide hybrid, and (ii) a signal generating portion; forming a complex comprising: (1) said analyte (A) complexed through said molecularly recognizable portion to (2) said recognizing portion of said entity (B); said entity (B) being complexed through said polynucleotide portion thereon to (3) said polynucleotide portion of said signalling entity (C); and detecting a signal by means of said signal generating portion present in said complex.</p> <p>4. The method of Claim 1 wherein the molecularly recognizable portion on said analyte comprises nucleic acid.</p> <p>7. The method of Claim 1 wherein said recognizing portion on said bridging entity comprises a polynucleotide sequence.</p> <p>16. The method of Claim 1 wherein said polynucleotide sequence on said bridging entity codes for a gene product or fragment thereof.</p> <p>20. The method of Claim 1 wherein said polynucleotide sequence in said bridging entity is covalently attached to another polynucleotide sequence.</p> <p>69. The method of Claim 1 wherein said recognizable portion on said analyte is a polynucleotide sequence, said recognizing portion on said bridging entity is a polynucleotide sequence capable of stably annealing thereto, said bridging entity is a single-stranded DNA polymer, and said step of detection by means of said signal generating portion on said signalling entity is based on non-radioactive detection.</p>
<p><u>Claim 53 of US5124246 (Count 4)</u></p>	<p><u>Claims 172, 173 and 179 of Pergolizzi's Amendment of June 3, 1985</u></p>
<p>A nucleic acid hybridization assay wherein: I. provided is a synthetic linear</p>	<p>172. A kit useful for the detection in a sample of an analyte having a molecularly recognizable portion thereon, comprising as</p>

<p>nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:</p> <p>(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and</p> <p>(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;</p> <p>II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;</p> <p>III. unbound multimer is removed;</p> <p>IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;</p> <p>V. unbound labeled oligonucleotide is removed; and</p> <p>VI. the presence of label bound to the multimer is detected.</p>	<p>components thereof:</p> <p>(i) a non-naturally occurring molecular bridging entity comprising a portion capable of recognizing said molecularly recognizable portion on said analyte; and a portion comprising a polynucleotide sequence; and</p> <p>(ii) a signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity polynucleotide portion, and a signal generating portion.</p> <p>173. The kit according to claim 172, characterized in that said bridging entity recognizing portion is selected from the group consisting of an RNA or DNA oligo- or polynucleotide sequence, an antigen, an antibody, a saccharide, a lectin, a hormone, a receptor, an enzyme inhibitor, a cofactor bonding site, an enzyme active site, and a receptor protein.</p> <p>179. The kit according to claim 172, characterized in that said signalling entity is selected from the group consisting of a single-stranded, double-stranded or partially double-stranded polynucleotide polymer, a naturally-occurring modified DNA, a polynucleotide polymer derived from a T (even) phage, a modified DNA carrying a cloned insert, a polymer derived from a filamentous phage, M13 phage or a variant thereof, and a polymer derived from a circular DNA molecule covalently attached to a non-radiolabelled signal generating portion.</p>
<p><u>Count 4 (Claim 53 of US5124246)</u></p> <p>A nucleic acid hybridization assay wherein:</p> <p>I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:</p> <p>(a) at least one first single-stranded oligonucleotide unit that is capable of</p>	<p><u>Claim 193</u> <u>of Pergolizzi's Amendment of Oct. 9, 1990</u></p> <p>193. A kit for the detection in a sample of an antigenic or biological analyte having a molecularly recognizable portion thereon, comprising as components thereof:</p> <p>(i) a container carrying a molecular bridging entity comprising a first portion capable of recognizing and binding to said molecularly recognizable portion on said analyte; and as second portion comprising more than one</p>

<p>hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and</p> <p>(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;</p> <p>II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;</p> <p>III. unbound multimer is removed;</p> <p>IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;</p> <p>V. unbound labeled oligonucleotide is removed; and</p> <p>VI. the presence of label bound to the multimer is detected.</p>	<p>polynucleotide sequence; and</p> <p>(ii) a container carrying a signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity second polynucleotide portion, and a signal generating portion;</p> <p>which molecular bridging entity and signalling entity form a detectable complex with said analyte, wherein the at least one component of said detectable complex is immobilized or the incorporation of the analyte into the detectable complex causes a detectable change in the signal generating portion thereof.</p>
<p><u>Claim 53 of US5124246 (Count 4)</u></p>	<p><u>Claim 196</u> <u>of Pergolizzi's Amendment of Oct. 9, 1990</u></p>
<p>A nucleic acid hybridization assay wherein:</p> <p>I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:</p> <p>(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and</p> <p>(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded</p>	<p>196. A method of detecting in a sample an antigenic or biological analyte having a molecularly recognizable portion thereon, comprising:</p> <p>forming a detectable complex comprising (a) said analyte, (b) a molecular bridging entity comprising a first portion capable of recognizing and binding to said molecularly recognizable analyte portion, and a second portion comprising more than one polynucleotide sequence, and (c) more than one signalling entity, each said signalling entity comprising a polynucleotide portion capable of annealing to and forming a stable polynucleotide hybrid with the polynucleotide sequences in said bridging entity second portion and a signal generating portion; and</p>

<p>oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;</p> <p>II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the analyte;</p> <p>III. unbound multimer is removed;</p> <p>IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;</p> <p>V. unbound labeled oligonucleotide is removed; and</p> <p>VI. the presence of label bound to the multimer is detected.</p>	<p>detecting said analyte by an amplified signal provided by said signal generating portions present in said detectable complex, wherein at least one component of said detectable complex is immobilized or the incorporation of the analyte into the detectable complex causes a detectable change in the signal generating portions thereof.</p>
<p><u>Claim 53 of US5124246 (Count 4)</u></p>	<p><u>Claims 194, 199, 209-213, 217-18 and 220 of Pergolizzi's Amendment of Dec. 22, 1992</u></p>
<p>A nucleic acid hybridization assay wherein:</p> <p>I. provided is a synthetic linear nonhomopolymeric nucleic acid multimer useful as a means for amplifying a detectable signal in an assay involving nucleic acid hybridization consisting essentially of:</p> <p>(a) at least one first single-stranded oligonucleotide unit that is capable of hybridizing specifically to a first single-stranded nucleic acid sequence of interest; and</p> <p>(b) a multiplicity of second single-stranded oligonucleotide units all of which are capable of hybridizing specifically to a second single-stranded nucleic acid sequence of interest that comprises a single-stranded labeled oligonucleotide, wherein the first single-stranded oligonucleotide unit is bonded directly or indirectly to the multiplicity of second single-stranded oligonucleotide units only via covalent bonds;</p> <p>II. the multimer is hybridized via the first oligonucleotide unit to single-stranded analyte nucleic acid bound to a solid phase or to a single-stranded oligonucleotide bound to the</p>	<p>194. A composition of matter comprising:</p> <p>a molecular bridging entity comprising a first portion capable of recognizing and binding to a molecularly recognizable portion on an analyte, and a second portion comprising a nucleic acid; and</p> <p>a universal signalling entity comprising a nucleic acid portion capable of annealing to and forming a stable polynucleotide hybrid with said bridging entity nucleic acid second portion, and a signal generating portion capable of providing, directly or indirectly, a detectable signal.</p> <p>199. The composition according to claim 194, wherein said analyte is selected from the group consisting of a nucleic acid and a protein.</p> <p>209. The composition according to claim 194, wherein said molecular bridging recognizing first portion comprises a nucleic acid.</p> <p>210. The composition according to claim 209, wherein said nucleic acid comprises an oligo- or polynucleotide.</p> <p>211. The composition according to claim 210, wherein said oligo- or polynucleotide comprises a modified oligo- or polynucleotide.</p>

<p>analyte;</p> <p>III. unbound multimer is removed;</p> <p>IV. single-stranded labeled oligonucleotide is hybridized to the multimer via the second oligonucleotide units;</p> <p>V. unbound labeled oligonucleotide is removed; and</p> <p>VI. the presence of label bound to the multimer is detected.</p>	<p>212. The composition according to claim 210, wherein said oligo- or polynucleotide is single-stranded or partially double-stranded.</p> <p>213. The composition according to claim 210, wherein said oligo- or polynucleotide is circular or linear.</p> <p>217. The composition according to claim 194, wherein said polynucleotide sequence in the molecular bridging entity second portion is linear or circular.</p> <p>218. The composition according to claim 194, wherein said nucleic acid in the molecular bridging entity second portion is single-stranded or partially double-stranded.</p> <p>220. The composition according to claim 194, wherein said polynucleotide sequence in the molecular bridging entity second portion is derived from a phage selected from the group consisting of a T even phage, a filamentous phage, an M13 phage, or a variant thereof.</p>
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